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NEUROPHYSIOLOGICAL PROCEDURES FOR THE DETECTION OF EXPLOSIVES

FINAL REPORT

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Summary

The aim of this project was to determine the feasibility of employing the rat as a biodetector for TNT. Studies were undertaken at NeuroCommunication Research Laboratories which explored the use of voluntary (operant) and involuntary (classical) conditioning techniques. The emphasis of this project was on the conditioning of an involuntary response: brain wave activity.[1] Once it was established that the rat could be utilized as a biodetector, steps were taken to find those classical conditioning and recording parameters which would mazimize detection capabilities. The final portions of this report include technical data which may prove to be useful to the researcher or applied scientist.

This report deals both with the general principles in the development of biodetectors and also with the specific, technical data collected in the studies. The first part of this report was written for the reader with no technical background in psychology, physiology, or electrical engineering.

Although this project was specifically directed toward the development of a biodetector for TNT, a similar biodetection system could be developed for any substance which produces sensory cues. The techniques described here have also been used successfully in the development of biodetectors for water contaminants. (Weinstein & Weinstein, 1978).

In general, the study was conducted over a four year period in three general phases.

Phase I. This was an exploratory phase during which rats were either trained to press a bar when subjected to the odor of TNT or had their brain waves analyzed prior to and during the stimulation with the odor of TNT or control odorants. The data clearly showed that of the 22 rats involved, 1 successfully showed the ability to press a bar when TNT was present, 19 showed predictable brain wave change to TNT but not to control odorants and 2 rats were trained to do both (press a bar and have brain wave changes).

Phase II. In phase II, 5 rats were classically conditioned to show brain wave changes only when TNT was present. All 5 were successfully trained.

Phase III. Eleven rats were intensively trained while their brain waves were recorded.

These data were subjected to intensive computer analysis (under a separate contract) and the preliminary reports (from Mr. Raymond V. Nolan of Fort Belvoir) indicate that the brain wave responses are clear indicators of the presence of TNT in contrast to various control odorants.

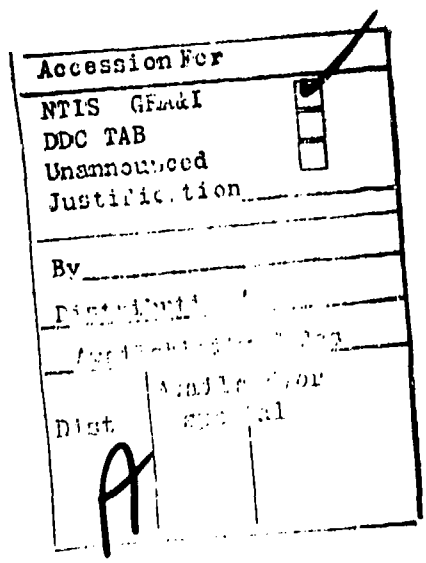
1. At the writing of this paper a new project has been undertaken to study the "rat biodetector" in applied field situations.

Foreward

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee or the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Acknowledgment

The authors gratefully acknowledge the contributions of Mr. Raymond V. Nolan of The U. S. Army Mobility Equipment Research and Development Command, Fort Belvoir, Va. in all phases of the research. Over the years Mr. Nolan frequently advised us on the conduct of the studies. His advice, always valuable, played a major role in the success we achieved in creating an explosives biosensor.



Background

The history of the use of animals (dogs, dolphins, pigeons, rats, etc.) by man for various purposes to supplement his armamentarium of detection, message transmission, etc., is long, and well documented. (See "The War Animals" by R. E. Lubow for an excellent description.) The use of these animals not only enhanced the detection abilities of man using his best equipment, but clearly made possible the performance of functions that could not have been accomplished by other means, regardless of the complexity or cost of the equipment.

Lubow supports the "biosensors" approach (animals used for the detection of mines, letter bombs and other explosives) by stating that "there is no system that can match their speed, sensitivity, and reliability." Similarly, after having reviewed the potential of dogs as mine detection systems, Nolan and Gravitte (1977) concluded that currently no known sensor or system of sensors exists which can approach the overall detection capabilities of a biosensor. Additionally, Mitchell (1976) described the training and use of dogs as mine detectors, and reported an accuracy level of approximately 90% in field condition studies.

Nolan and Gravitte have also reviewed the use of other animals in biodetection systems. These ranged from larger animals, such as the deer and wolf to smaller animals such as the fox and the ferret. Although these animals may have an acute olfactory sense, there are problems of compatibility with man. Insects have also been considered because of their acute olfactory sense, however, they are difficult to interface with a human-based system. In addition, insects are highly genetically preprogrammed, which makes them difficult (if not impossible) to train and allows them to be distracted by other olfactory cues which "trigger" behaviors such as feeding and reproduction.

The laboratory rat was not initially considered a candidate for the biodetection system, primarily because it could not be easily used in the same manner as the dog and other relatively large animals. That is, it is not feasible to place a leash on a rat, allow it to survey an area, and wait for an overt behavioral response in the field (such as sitting or pointing). However, if the rat is placed in some type of portable containment, it could be carried to the area to be surveyed and air and/or soil samples would be delivered into the containment. (see Fig. 1) In general, the laboratory rat can be easily handled and trained (indeed, much of what is known today about learning processes was derived from studies with the rat) since it has been bred specifically for those purposes.

Advantages of the Rat as a Biodetector

Although the dog has been well-established as a biodetector for target substances, the rat was considered as an alternative because of the potential advantages listed below.

1. Size. Rats are smaller and lighter than dogs (750 to 1 magnitude difference. Therefore, the entire rat biodetection unit can be contained in an enclosure such as an attache case. (see Fig. 1)

2. Maintenance. Rats occupy smaller cages than dogs. The Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 73-23, Revised 1972) states that dogs weighing in the range from 33 to 66 lbs. need a cage of about 36 cubic feet; whereas, a rat weighing over 0.66 lbs. needs only 0.16 cubic feet. This ratio of 185:1 in volume needed for living space is similarly reflected in food required and waste produced by the two species. Also, unlike dogs, rats may get all their exercise in their home cage.

3. Training. Rats can be trained routinely and with limited human intervention. (One trainer with the aid of electronic equipment can simultaneously train several rats). The dog requires an individual trainer who is also required to handle the dog in all field detection operations.

4. Field Implementation. Because of the size, training and maintenance factors mentioned previously, one handler can monitor several rats simultaneously. This is an important asset because it allows the use of "rotational" and "consensus" systems for detection. A rotational system would allow some rats to be "on duty" while others were "off duty" without removing the rats from the monitoring chamber. This procedure can be accomplished by the use of discrimination cues (e.g., light or sound in the chamber) which initiates or suspends the detection behavior in the rat. This procedure would minimize fatigue effects and allow continual monitoring.

A consensus system could greatly enhance the accuracy of detection. Because several rats could be monitored at the same area easily, a "poll" of the responses could be taken, allowing a more accurate probability statement to be made concerning the presence or absence of the target substance. Although both the rotational and consensus systems could be implemented with dogs, the many potential difficulties would most probably make it cost prohibitive.

5. Noise and Appearance. Rats, even when provoked, make little noise and can be easily concealed, whereas dogs are difficult to silence and conceal. The concealed rat is not socially intrusive in crowded areas such as airports, and moreover, offers no overt clue that a particular area is being monitored for explosives, contaminants, etc. This makes the task of the terrorist or other offender more difficult.

6. Invasive Physiological Procedures. The use of invasive physiological techniques, such as electrical brain stimulation (EBS) used as a "reward" for the correct response and brain wave recording used as an "indicator" of the target odorant, have been standardized and are routinely performed on rats.

Not only are the "reward" and "indicator" techniques more complicated and less standardized in the dog, but public opinion seems to be directed against scientific applications for animals which are routinely used as house pets.

7. Signalling the Presence of the Target Substance. The detection of TNT has been signalled by either a bar press or by characteristic brain wave patterns in the rat. Dogs have been trained to exhibit gross responses, such as sitting or pointing, to indicate the presence of the target substance.

This type of gross response becomes not only impractical in some situations but, more importantly, may be ambiguous. This potential ambiguity restricts accurate detection of target substances by dogs to situations in which this type of gross response can be emitted without outside interference from humans, other animals or physical barriers.

The rat, because of its size and the manner in which the response is transmitted, does not have to be physically visible and therefore can be placed in physically restricted areas where a dog could not enter. Furthermore, the use of air sampling tubes can permit the odorous target to be sampled on-line from regions which dogs could not easily reach, such as the upper regions of aircraft, high shelves and lockers, etc., but which can routinely be used with rats housed in small portable boxes.

8. Overall Costs. Rats are less costly than dogs in the following areas:
    1. Initial investment: (dog - $250.; rat - $ 5.00)
    2. Food: (rat weighs 100 times less)
    3. Medical Care: (rats are highly immune to infection)
    4. Housing and Maintenance: (rats require 180 times less living volume)
    5. Training: (rats may easily be trained by electronic machine)
    6. Transportation: (rats are smaller and lighter)
    7. Handlers: (rats will work for anyone; not so the dog)

Lubow (1977) estimated that the cost to train a dog in his studies was over $10,000. per dog.

The cost of the production of a rat biodetector could be substantially less; possibly under $500. per rat, total cost, when all procedure become optimized. Thus, an estimated 20:1 cost-ratio advantage exists for the production of rat biodetectors over dogs. This 20:1 initial cost advantage for the rat, added to the other advantages of the rat including the low maintenance and field use costs of the rat combine to make it the obvious choice for biodetection development.

General Principles in the Development of a Biodetector

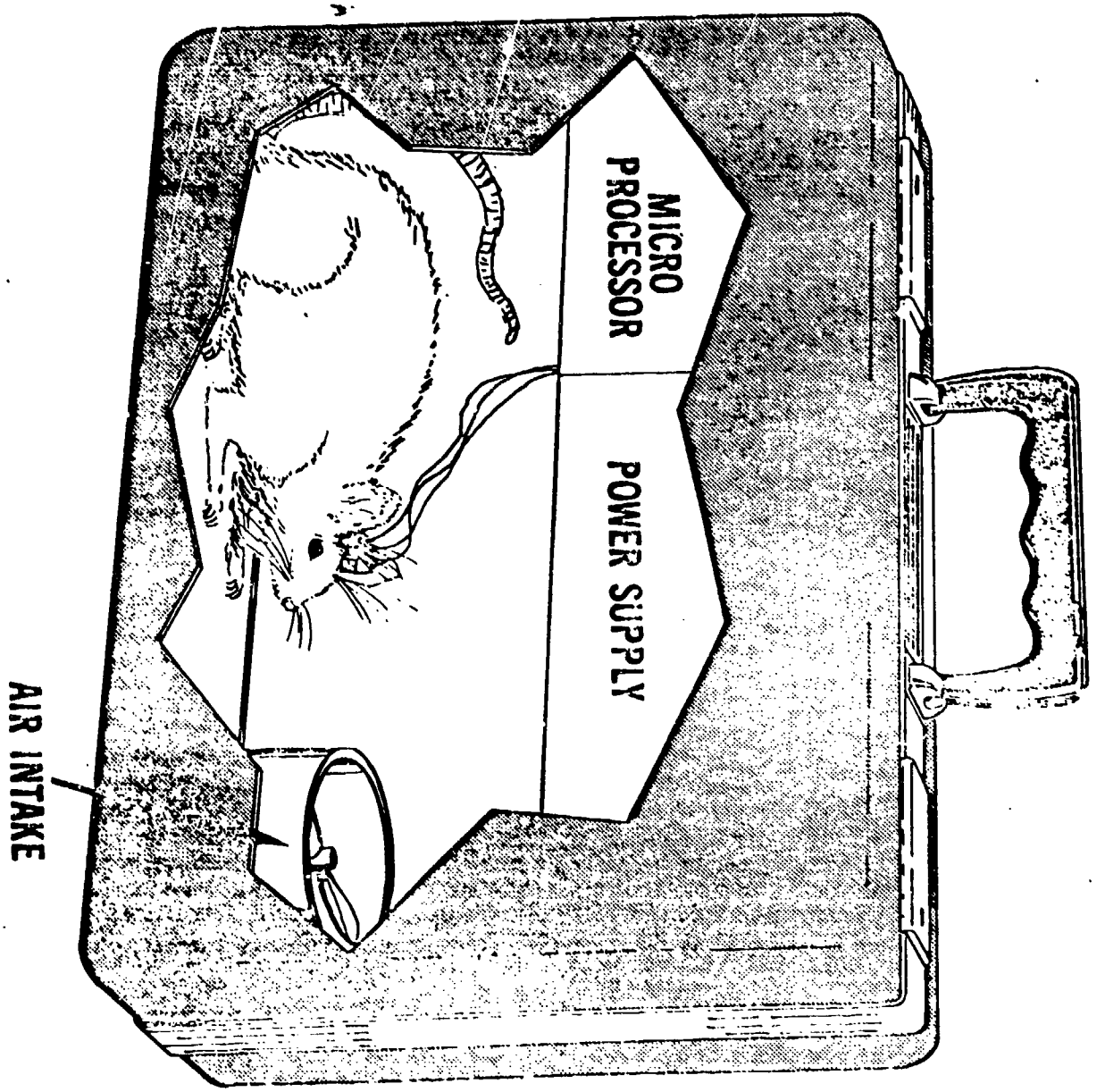
The following section was written to acquaint the reader with some of the basic principles in psychology and neurophysiology which have been used in the development of biodetectors at NeuroCommunication Research Laboratories. The literature in these areas is extensive; however, the discussion here will focus only on aspects directly related to the present series of studies.

Learning or conditioning is generally defined as a relatively permanent change in behavioral potential which results from experience. The two basic subtypes of learning which were employed in the present studies were classical conditioning and operant conditioning. These two basic processes are believed to be fundamental components of the behavioral repertoire of most organisms. Although there is currently some debate over the theoretical distinction between the two types of conditioning, the paradigms which produce their effects are well established. Both types of conditioning have been used successfully and have taken on complementary roles in maximizing the development of the rat as a biodetector.

Principals of Conditioning

Conditioning is a set of procedures which may be used to teach without the use of language. It relies upon the fact that animals make temporal associations of stimuli. Thus, if two events occur which are closely ordered in time, there is a strong tendency to consider the first event as the cause of the second. For example, if one observes a flash of lightning followed quickly by a power failure, then one would be likely to blame the lightning for the power failure.

FIGURE 1



(Though, of course, the power failure need not be caused by the lightning.) In this same manner, if two events happen in a short period of time, then an observing animal acts as if the first event causes the second. In the case of rats learning to detect TNT, many such temporal pairings of TNT odor to a specific event are necessary before the rat behaves as if the TNT odor causes the specific event.

More details of our use of classical and operant conditioning are explained in subsequent paragraphs, but in general:

(1) Operant Conditioning requires the subject to produce the first event of the temporally paired events. If the subsequent, second, following event is desirable to the rat, then the rat will produce more first events.

(2) Classical Conditioning requires a reflex to exist prior to the conditioning. The second event is that which naturally causes the reflex, and the first event comes to elicit the reflex upon successful conditioning. (see Fig. 2) Classical conditioning involves the acquisition of relexive, involuntary responses. In the present studies, this response comprised involuntary brain patterns which can be measured by the electroencephalograph (EEG) recorded directly from the rat's cortex. This involuntary response (unconditioned response) can be elicited by a stimulus named the unconditioned stimulus. For example, placing food in the mouth produces automatic salivation, a puff of air to the eye causes reflexive eyelid closing, and a loud auditory stimulus produces a number of reflexive autonomic nervous system responses such as changes in heart rate, and skin resistance.

At the turn of the century, Pavlov described what has become known as classical conditioning. He found that, after repeated temporal association of a (conditioned) stimulus with a neutral stimulus (one which previously did not produce the unconditioned response), the previously neutral stimulus became a conditioned stimulus which would also elicit the unconditioned response. This process is diagrammed in Fig. 2.

The paradigm used in the present study follows a procedure similar to the one described above. The unconditioned stimulus, UCS, is an electrical stimulus which is delivered to a "pleasure center" of the brain, the medial forebrain bundle (the ratiionale for the use of this electrical brain stimulation (EBS) technique will be discussed later). This stimulation produces a class of unconditioned brain responses which can be measured by certain characteristics of the EEG. The conditioned stimulus in this case is the presence of the odor of TNT. The repeated temporal pairing of the TNT odor with the EBS results in TNT acquiring the potential to elicit the characteristic EEG response by itself.

The above paradigm also leads to the possibility of anticipatory response conditioning instead of, or in addition to "pure" classical conditioning. This procedure may, in practice, include operant components. However, the aim of the present studies was not to examine the components which make up the conditioning process, but to explore the feasibility of the technique in the detection of explosives and to set up a paradigm which maximizes the detection process.

The advantages of using the classical conditioning paradigm in the development of biodetectors are as follows:

1. There is an elimination of a voluntary response on the part of the rat.

Figure 2

Paradigm of Conditioning

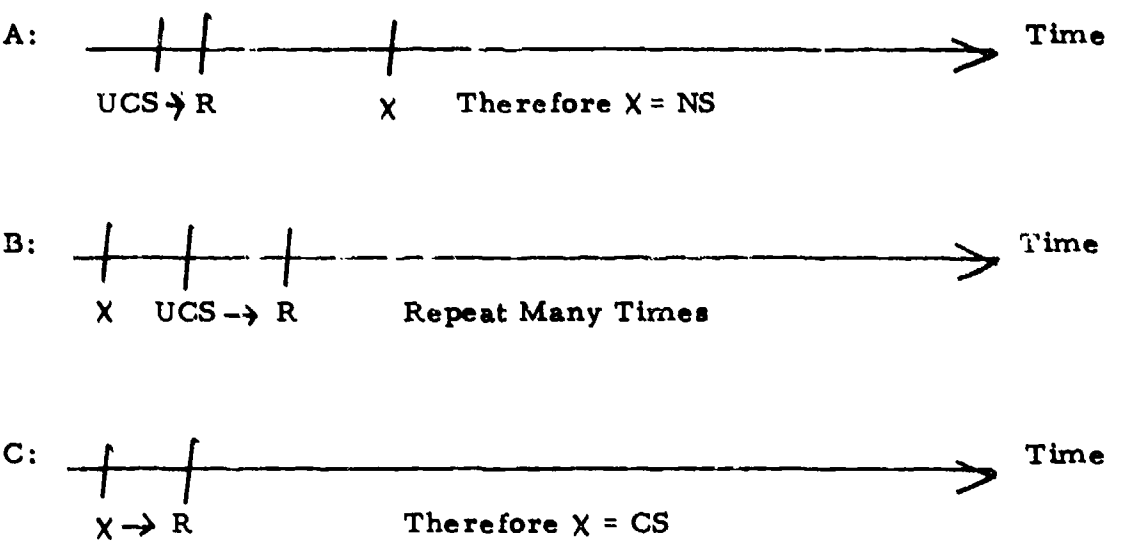
Conditioned Stimulus = CS

Unconditioned Stimulus = UCS

Neutral Stimulus = NS

Stimulus = X

Response = R



This minimizes the number of "false-alarms" since the animal has no overt control over the delivery of the reinforcer.

2. The brain response produced by the classical conditioning is an analog response. That is, the characteristics of the cortical frequency spectrum of the EEG, rather than a digital (yes/no response) on the part of the rat, indicates the presence of the target odorant. This technique may permit the detection of smaller quantities of TNT.

3. The latency between the presence of the odorant and the production of a response is very brief. A voluntary response by an animal requires additional, although only slightly greater, processing time.

4. Environmental distractors, such as other animals, noises, etc. are less likely to affect an involuntary response.

Disadvantage. The major disadvantage with the classical conditioning paradigm lies in the implementation of the necessary electronic instrumentation. With the recent advent of miniaturized electronics and microprocessors, however, this does not seem to be a serious shortcoming.

Operant Conditioning.

While classical conditioning applies to involuntary responses, operant conditioning requires the animal to make a "voluntary" response. If the response results in something which is needed (or "liked") by the organism, the frequency of that voluntary response will increase (reinforcement/reward). On the other hand, if the voluntary response results in something which is not liked by the organism, the frequency of this response will decrease (punishment). The present discussion will be limited to positive reinforcement since it was the technique employed in the present studies. The use of aversive contingencies (negative reinforcement and punishment) was avoided since their response patterns tended to be less stable than those which result from positive reinforcement contingencies.

The scope of operantly conditioned behaviors can be seen by examining dog training techniques, personnel management, educational programs, language acquisition in humans and the traditional bar pressing of the laboratory rat.

The behaviors emitted in operant conditioning may not be initially elicited directly by a stimulus, as in the case of classical conditioning. Environmental factors modify or "shape" the behavior of the animal in the desired direction. In the case of the development of a biodetector, the trainer controls the environmental factors. This is accomplished through a process called "shaping" during which approximations of the desired response are reinforced. For example, if the desired behavior is a bar press in the rat, the first step in the shaping procedure would be to reinforce orientation toward the bar. A second step would be to reward proximity to the bar. A third step may reinforce bar touching, while waiting for the final stage when pressure on the bar is sufficient to close a mechanical switch. Once an approximation is achieved it is usually not beneficial to reinforce earlier approximations unless there is a prolonged period of nonresponding. This technique allows a rat, who possesses an acute olfactory sense, not only to be trained to detect the presence of a particular target odorant, but in addition, to be trained to detect sequentially smaller concentrations of the odorant.

In the present study, shaping of operant behaviors was used in a role complementary to classical conditioning. The parameters of the medial forebrain bundle electrode implantation (reinforcement center) were assessed by stimulating the site when appropriate behavioral approximations to the final desired response were emitted by the animal. In this way, maximal stimulating conditions, that is, current levels and latency and frequency of stimulation could be determined by recording the number of bar presses at various stimulation levels.

In addition, operant shaping techniques were used to restrict the animal's movement and to insure it was in the proper position relative to the odorant source for maximal olfactory stimulation during the classical conditioning phase of the studies. This goal was achieved by reinforcing the rat with the electrical brain stimulation only when it was motionless and had its nose placed in the air delivery path.

Reinforcement Schedules

The question arose in the development of biodetectors as to the number of times the animal needs to be rewarded for a correct response. This is an especially important consideration when testing in the field, where reward for correct response is not always possible. It is also generally agreed among behavioral scientists that animals perform at maximal levels when not rewarded after every correct response. This procedure, called intermittent reinforcement, in fact, strengthens the response, so that it is less likely to be forgotten over time (extinction). The procedure employed in the present studies shifted the rat to an intermittent reinforcement schedule once the behavior was well established. This procedure has produced animals which were resistant to extinction for periods up to three months between practice sessions. The maximum length between practice sessions which is consistent with resistance to extinction has not yet been determined.

Advantages of Operant Techniques

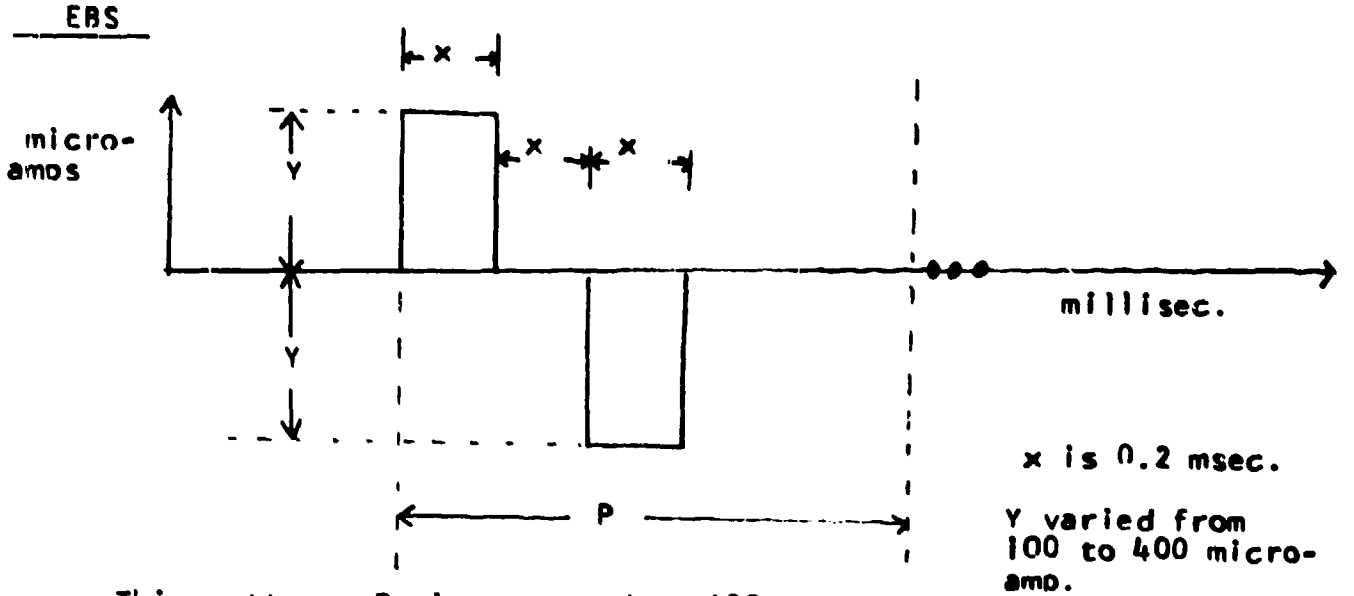
1. In addition to having a supportive role in the classical conditioning of the EEG, operant techniques can be used independently in the development of biodetectors. The main advantage of the operant technique lies in the ease of implementing the biodetector system. With relatively little equipment, an efficient system can be developed.

2. As mentioned previously, a shortcoming of the operant technique is the need for the animal to produce a voluntary response. This procedure may result in a higher proportion of "false alarms" since the rat is anticipating reinforcement

Although the sole use of punishment does not result in efficient training, it has been found that mildly aversive stimuli used along with reward can enhance training and minimize errors. This combined procedure leaves the alternatives for the correct response to be rewarded while the incorrect response produces a condition which although not liked by the animal, is not overly stressful. Presently, a study at NeuroCommunication Research Laboratories, Inc. is underway which will try to determine the best technique or combination of operant techniques to maximize biodetector development.

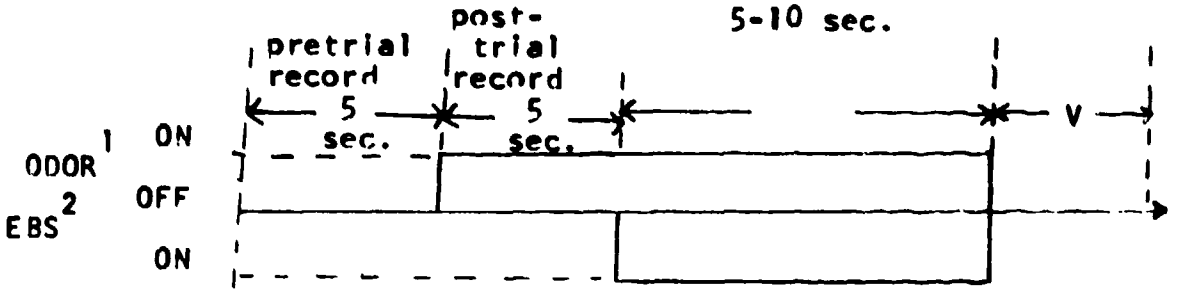
Figure 3

Training Parameters for Electrical Brain Stimulation (EBS)



This pattern, P, is repeated at 100 times per second for 100 to 500 msec.

Training: Two 30 minute periods of training per day.



1: Odor may be one of four including TNT.
2: EBS is available only for stimulation with TNT.

Neurophysiological Principles

"Pleasure" Centers. When miniature electrodes are implanted in the medial forebrain bundle (MFB) of the brain, a rat will repeatedly press a lever which causes a small current to be applied to this site (Electric Brain Stimulation, EBS). The discoverer of this phenomenon, James Olds, believed that stimulation of the MFB and other self stimulation sites produces an artificial activation of the brain's normal reinforcement mechanism. The most striking characteristic of the self-delivered electric brain stimulation is that it seems to be insatiable. Our rats have produced self stimulation rates exceeding 4/sec.; 3,000 responses per hour was not an uncommon rate. These continuous high rates of responding cannot be found in a healthy rat responding for food or drink.

EBS of the MFB was used not only as the reinforcement for the operant phases of the present studies, but also as the unconditioned stimulus in the classical conditioning phase. (see Fig. 2) The advantages of EBS over those of other types of reinforcement are as follows:

1. Does not satiate
2. Does not require prior drprivation, as is needed with food or water, it therefore lessens the probability of nutritional deficits, and resultant malfunctioning of the brain or receptors.
3. Provides a strong motivation without interfering with behavior.
4. Less likely to be distracted from EBS by food, water, estrous females, etc.
5. Allows a more stable level of reinforcement, that is, the value of the reinforcer does not change as more are presented.
6. The latency of reward is only in milliseconds, whereas food, water, etc. have much greater latencies.

The disadvantages of EBS: A surgical operation is required (equipment and two hours time). Some rats may require "priming" after certain periods of delay between "detection" sessions. That is, the rats have to be "reminded" of the reinforcement. (This problem is easily overcome by presenting a few "free" stimulations prior to beginning the session.)

Recording Technique

As mentioned previously, when the classical conditioning procedure was used in the development of a biodetector, an "involuntary" response on the part of the animal signalled the presence of TNT. Changes in the electrical activity of the brain were used as the conditioned responses to the TNT. The electrical activity of the brain, the electroencephalogram (EEG), has been found to be a correlate of many behavioral states.

The EEG, as recorded from over the cortex, is a time varying voltage which seldom exceeds 100 μV and is usually recorded in the frequency range of 0-40 Hz. Since all integrative behavioral activities (such as conditioning a rat to detect TNT) are mediated by the brain, it was believed that measuring the EEG before, during, and after conditioning would be the most objective and direct means of determining the presence of the target odorant.

Various EEG recording sites were attempted. Transcortical (anterior left to posterior right), cingulate, parietal, occipital, hypothalamic and frontal recording sites were attempted at various times.

Computers have been utilized in the present study to analyze objectively the EEG data during the various stages in conditioning. Various types of spectral analyses, such as the zero-crossing and fast Fourier transform were employed to determine frequencies. Other methods such as correlational analysis can be used to determine the degree of relationship between segments of the EEG. Correlations can be determined between electrode sites and over time. (i.e., cross correlation and autocorrelation).

The objective of the final phase of the this project was to determine the best EEG index of TNT conditioning and also to determine which of the various computer analysis techniques best extracts this index from the data. The ultimate goal is to develop a self contained biodetecting unit (see Fig. 1) in which the conditioned EEG response could be analyzed by a microcomputer very rapidly (within seconds) and do this in an ongoing manner. Because of its small size, a unit of this design would be greatly flexible for various field uses.

The EEGs in the final phases of this study were recorded with a filter setting of .03 - to 300 Hz, -3dB points. Although this is not a conventional bandwidth used with EEGs, ideal recording conditions permitted this flexibility. Preliminary results on these data suggest that the recording of these additional frequencies can enhance the detection of TNT. Indeed, intensive computerized analyses of the EEG during TNT and non-TNT stimulation has already shown clear differences and the analyses are being continued by Raymond V. Nolan.

Overview of Training Procedures and Results

The general background and principles which apply to the development of rat biodetectors have been discussed above. The following section will review the specific procedures used to condition rats operantly and classically to detect TNT in the studies conducted at NeuroCommunication Research Laboratories from 1975 to 1979.

This project was divided into three phases. The preliminary phase employed both operant and classical conditioning. The data for 23 individual subjects for the phase one classical conditioning can be found in Appendix 2. The two final phases of this project were restricted to classical conditioning. The data for phase two (N=5) is summarized in this section. The objective of this phase was to isolate the recording site which most readily reflects EEG conditioning. Phase three was directed at optimizing EEG analysis and recording techniques. (See Table 1 for Summary of Self-Stimulation Parameters and Recording Sessions. See Table 2 for Summary of Phase 2 Statistical Data.) The sample of nine rats were classically conditioned and their brain waves were recorded with a wide bandwidth (300 Hz) under ideal conditions. These EEGs were subjected to a number of analyses, done by an independent firm under another contract for the U. S. Army Mobility Equipment Research and Development Command, Fort Belvoir, VA., in order to isolate the EEG spectral frequencies and analysis techniques which most readily determine the presence of target odorant.

Table 1

Summary of Self-Stimulation Parameters and Recording Sessions *

C0020 ID	Other ID	1	2	3	4	5
A	64	63	300	300	<5	6
B	--	187	250	300	< 5	7
C	72	64	250	350	<5	5
98	E	106	200	200	2	3
97	F	64	300	200	2	3
G	81	44	200	550	<5	4
100	--	170	200	150	2	3
103	--	110	200	150	2	3
104	--	170	200	150	2	3

* includes only rats with established self-stimulation rates

1 - maximum mean rate per minute mean of all subjects recorded

= 108.6 per min.

2 - microamp level

3 - duration in msec.

4 - number of self-stimulation training sessions

5 - number of recording sessions

C-0020 ID: original identification code of rat

Other ID: another code used to describe rat

At the time of this writing, Raymond V. Nolan has conveyed to us that the results look very promising.

Electrode implantation. Rats were chosen for the surgical procedures on the basis of their weight (at least 250 gm), physical condition, and sex (male). Only on rare occasions were rats not considered suitable at this stage. Respiratory problems and hypo- or hyperactivity levels were some factors which deferred rats from surgery.

All surgery was performed under general anesthesia (Chloropent ^{R}) in a clean environment. A totally sterile operating environment is not required for surgical procedures with rats, as they are highly resistant to infection. The surgical procedure involves the following steps: (see Cooley and Vanderwolf, 1978 for good description).

1. The rats received an intraperitonal injection of anesthetic according to body weight.
2. The rat was prepared for incision by shaving its head and applying an antiseptic to the scalp.
3. The (anesthesized) rat was placed into the stereotaxic instrument which holds the head stationary by means of a bite bar and ear bars.
4. A longitudinal incision was made with a sterile scalpel.
5. The skin was retracted and landmarks on the skull were located.
6. Standard coordinates from a stereotaxic atlas are used in conjunction with the individual landmarks to determine the specific brain sites.
7. After the coordinates for the medial forebrain bundle (MFB) and for the recording electrodes had been marked on the skull, small holes were drilled at these sties.
8. The electrodes used for stimulating the "pleasure center" (MFB) were inserted into the brain to a depth specified by the atlas.
9. The cortical recording electrodes, placed at occipital, parietal and cingulate sites, were threaded into the skull.
10. The MFB and recording electrodes were connected to a small plug.
11. The electrode plug was then cemented onto the skull with cranioplastic (dental) acrylic.
12. The incision was cleaned, sutured and antibiotic cream was applied.
13. The rat was placed in a heat-regulated chamber until the anesthetic drug were off.
14. Complete recovery was usually within a one week period.

Self-Stimulation

In order to determine the correctness of the electrode placement in the MFB, after recovery from surgery, the rat was placed in a Skinner box (training cage) containing a bar attached to a microswitch. When the bar was pressed, a stimulus was delivered to the MFB.

"Shaping" is the procedure according to which the rat learns to press the bar. This is done by a method which first stimulates the rat if it faces the bar, then if it approaches it, and finally only if it presses it. If the rat becomes a "self-stimulator," we know that the electrode was well-placed.

Cortical Frequency Spectra (CFS) Recordings. Following determination of the optimal characteristics of the EBS, we recorded the CFS of all rats from all four sites prior to any conditioning procedures. The signals from the brain were amplified (Grass Polygraph, Model 7) and fed to a PDP 11-34 Computer, programmed to compute a CFS from each site. The Fast Fourier transform (FFT) was employed in this analysis. The seven EEG bands analyzed were: (1) 1.3.5;; (2) 3.51-7.5; (3) 7.51-12.5; (4) 12.51-19.5; (5) 19.51-25.5; (6) 25.51-35.5; and 35.51-45 Hz. In addition to the four CFSs, the computer also computed cross power spectra across the occipital and parietal and across the parietal and cingulate sites. For each determination (i.e., band x site) we also calculated the standard deviation, which enabled us to compute "t-tests" between these "preconditioned" recordings and the recordings made during TNT or control stimulation.

Stimulation with Odorants. Prior to stimulation with TNT, there were 60 "sham" trials without odorant stimulation, enabling us to acquire "pre-conditioning" control data from all sites. On the first day of stimulation with TNT to initiate conditioning, the activity was recorded for 60 trials (30 TNT and 30 control trials) randomly ordered by the computer. 5 sec of CFS prior to stimulation (with TNT or control was recorded. Then 10 sec of odorant stimulation was given while recording CFS during the first 5 sec. EBS (if TNT was used) or no EBS (if control odorant was used) was delivered at that time.

Analyses. There were three basic forms of analysis: (1) comparison of the CFS obtained during baseline brain response (BBR) versus the TNT vapor brain response, (2) the immediate pre-TNT-brain response versus the TNT vapor brain response, (3) the TNT vapor brain response versus the control-brain response. There were large numbers of statistical comparisons which comprised: 4 brain recording sites, 7 EEG bands, repeated sessions, baseline-brain recordings, 30 trials of TNT stimulation, 30 trials control stimulation, "early" vs "late" trials (e.g., the 3rd and 4th trial per session versus 58th and 59th trial per session.

Results

Conditions. All five rats proved to demonstrate changes in their CFS to the persence of TNT which differed from: the baseline brain recordings, the recordings immediately prior to the TNT stimulation, and/or the control stimulation. Some rats showed all forms of change, others showed changes for some of the comparisons. (see Table 2)

Sites. Some rats showed pervasive changes in all sites to TNT stimulation: others showed changes which were more restrictive in locus. In general, the most effective site, i.e., the one which showed changes most consistently and most frequently was the cingulate gyrus.

EEG Bands. In general there was a fairly pervasive change among the seven frequency bands studied. However, the most consistent and most frequent bands to show changes were bands 1-2, 6-7 (i.e., 0.5 to 7.5 Hz and 25.6 to 45.5 Hz), or the so-called Delta, Theta, Gamma 1, and Gamma 2 bands, and less so for the Alpha and Beta bands.

Table 2

Rat # 45 Post Conditioning T-Tests

Key to Symbols

- $ - Post Explosive differs from pre norms
- & - Pre-Explosive differs from pre norms
- @ - Pre non-Explosive differs from pre norms
- # - Post non-Explosive differs from pre norms
- E - Pre Explosive differs from Post Explosive within the post 60 trial session.
- P - Post non-Explosive differs from Post Explosive within the post 60 trial session.

Significance Level = 0.001

Minus (-) Sign indicates an increase over pre-norms for $, &, @ and # and a decrease from post-explosive for E and P

Band and Site Description

Band	Frequency	Site	Site
1	1-3, 5 Hz	1	Occipital
2	3.51-7.5 Hz	2	Parietal
3	7.51-12.5 Hz	3	Cingulate
4	12.51-19, 5 Hz	4	M.F.B.
5	19,51-25.5 Hz	5	Cross Power (Sites 1 + 2)
6	25.51-35.5 Hz	6	Cross Power(Sites 2 + 3)
7	35.51-45 Hz		

	Site					
Band	1	2	3	4	5	6
1	@ # & -E	@ & -E	-$ -S-E	@ -$-E-P		
2	@ # & $	@ # & $	-# -$-E	&	@ &	
3	@ # & $-E-P	@ # & -E-P	-$-E	& -$-E-P	@ &	
4	@ # & $ -P	@ # & $ -P	-E	@ # & -E-P	@ &	
5	@ # & $-E-P	@ # & $-E	-E	@ # & -E-P	@ &	
6	@ # & -E-P	@ # & -E-P	-$-E-P	@ & -E-P	@ &	
7	@ # & -E-P	@ # & -E-P	-$-E-P	@ & -E-P	@ &	

Table 2 (continued)

Site

Rat # 51

Band	1	2	3	4
1				
2				-$
3				-$
4				
5				
6				
7			-& -$	

Site

Rat # 54

Band	1	2	3	4
1			-$	
2			-$	
3		@ # & $		@ # & $
4		@ # & $	-@-# -$	@ # & $
5		@ # & $	-$-E	@ # & $
6		@ # & $	-$-E	@ # &
7		@ # & $	-E	

Table 2 (continued)

Site

Rat # 55

Band	1	2	3	4
1	-$-E-P	-$	-$-E-P	-$-E-P
2	@ # & -E-P		# -$-E-P	@ # -$-E-P
3	@ # & -E-P		@ # & -$-E-P	@ # & -$-E-P
4	@ # &		@ # & -$-E-P	@ # & -E-P
5	# &		# -E-P	# & -$-E-P
6	# &		@ # & -E-P	-$-E-P
7	&		& -E	-E-P

Site

Rat # 56

Band	1	2	3	4
1	$		@ # & -$ -P	-$
2		-$	@ # & -$ -P	-$-E-P
3	@		@ # & -$-P	-$-E-P
4	@ # & $		-@-#-& -$-P	-P
5	@ # & $ E	E	-@-#-& -$ -P	
6	# $	-&	-$ -P	-$
7	# -E-P	-@-#-& -$	-$ -P	-$-E-P

The procedure also helps us to determine the ideal parameters of the current. That is, by continually modifying the voltages, and relating them to the resultant rate of bar pressing, we determined the ideal parameters for that rat.

The stimulation parameters were determined by recording the number of times the rats pressed the bar under different intensities and durations of stimulation. The maximal stimulation levels for a particular rat were, therefore, the ones which produced the highest frequency of bar-presses per minute.

Odorant delivery system. The TNT and control odorants (such as grass, asphalt, woodchips, etc.) were presented to the rats by means of an air delivery system. Filtered breathing air was passed over separate containers of loosely packed TNT granules or various control substances. The tubings which carried the odorants were kept separate until they reached an opening in the Skinner box. Random presentation of the odorants were controlled by modular logic units which activated solenoid values. When a TNT trial was presented, air was passed only over the TNT granules while a slight vacuum was applied to the control odorants. This procedure was reversed during the control trials. All exhausted odor-laden air was continuously evacuated from the building by means of exhaust tubes placed in the test chamber. This procedure prevented any significant build-up of TNT in the laboratory, thus minimizing the possibility of false stimuli.

Operant Conditioning

Training Procedure. The rat was trained, by means of shaping, to discriminate between the TNT and control odorants. If the bar was pressed during the presentation of TNT, the animal was "rewarded" with EBS. However, a bar press during a control trial did not yield a reward. Additionally, an incorrect response also resulted in increased time between the last incorrect trial and the next trial. "Time-out" means that there is an additional delay in the possibility of receiving another EBS.

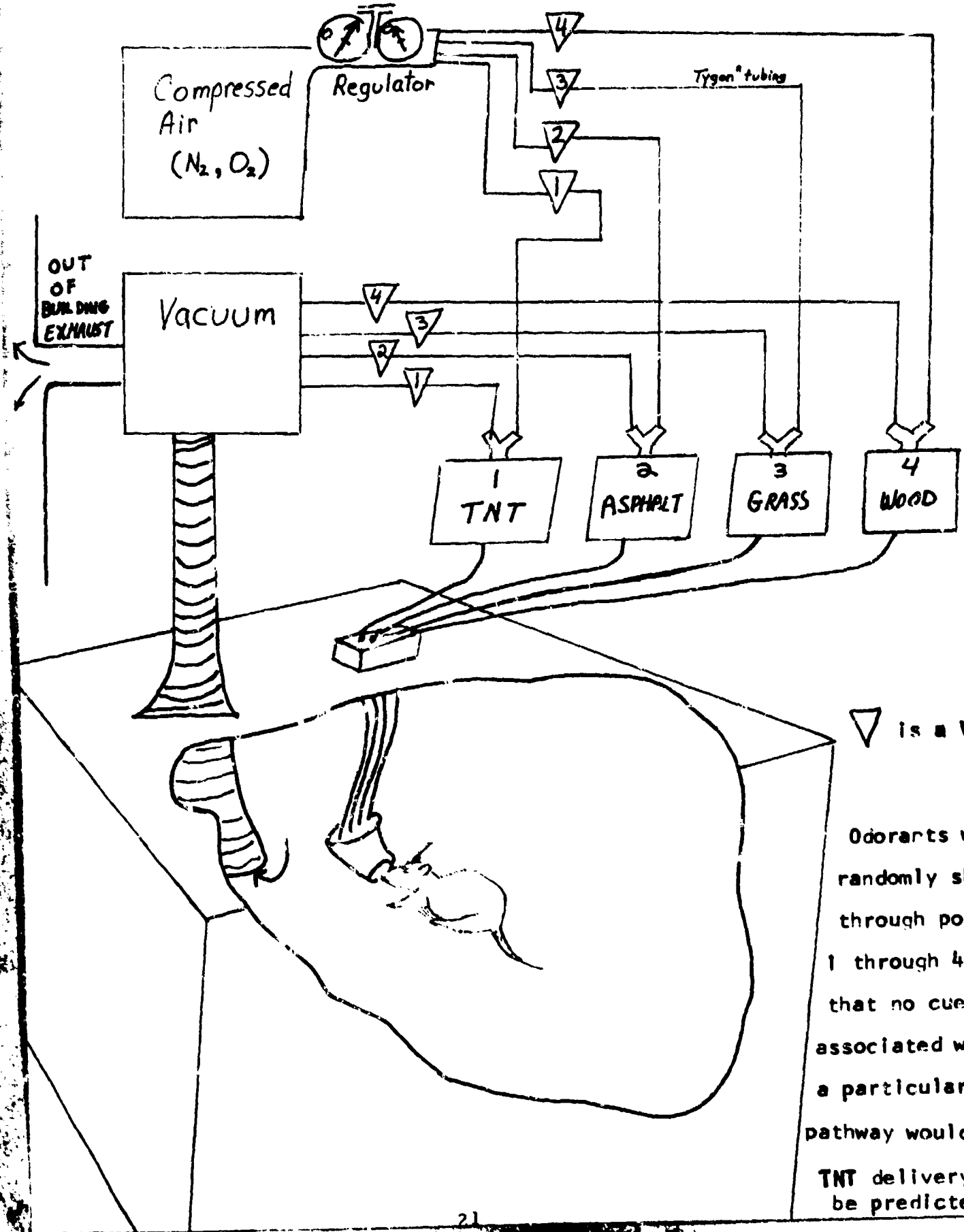
After a consistent response level was achieved, the rat was shifted to an intermittent reinforcement schedule. As mentioned previously, an intermittent reinforcement schedule is more resistant to extinction (forgetting). Therefore, not every bar-press during the presentation of TNT was rewarded with EBS.

Recording Chamber

In the final phase of this project, a specially designed chamber was used to insure the best possible environment for classical conditioning and brain wave recording. The chamber was constructed with double-layered copper sheets or screening placed between the interior and exterior walls (Faraday Box, see Appendix 2 for technical description). This type of chamber greatly reduces the degree to which outside electrical signals (e.g., power line and machinery signals) can contaminate the recordings of the small EEG signal. In addition, the chamber was sound-attenuated and specially ventilated. A custom designed testing animal restraint cage which minimized excess movement on the part of the test subject was coupled with the air delivery system. The EEG preamplifiers and this testing cage were both enclosed in the Faraday Box.

FIGURE 4

AIR FLOW DELIVERY SYSTEM



The inside of the chamber was of modular construction so that side and wall Formica panels could be removed and washed with a TNT solvent prior to each testing session. This procedure minimized the possibility that residual odorants in the chamber would interfere with conditioning the rat.

Classical Conditioning

The major focus of the project was on the classical conditioning of brain wave activity of the rat to the presence of TNT. The EEG was recorded on both TNT and control odorant trials under the following conditions:

1. pretraining trials
2. pre TNT or Control onset
3. post TNT or control onset

A number of variations on the length of the EBS, interval between TNT and EBS, intertrial intervals and frequency of TNT to EBS pairing were explored. The parameters shown in Fig. 3 tended to be the most useful.

The TNT and control odorants were presented by an air delivery system similar to the one described previously. A pseudo-random order generator activated the air delivery system with somewhat equal numbers of presentations of TNT and control odorants at first. The EBS which was paired with the TNT was presented at the same rate which matched the rate that the rat produced in the self-stimulation session.

Paradigm Summary

Surgery comprised placing an electrode in the Medial Forebrain Bundle area and recording screws in the skull. After recovery, the rat was taught to self stimulate. If a rate of over 10 per minute was achieved, the rat was exposed to operant, or classical conditioning. Often, it was necessary to shape the rat to keep its nose near the place where the odorants were emitted.

In the operant conditioning paradigm, the rat was rewarded with a short period of self stimulation during TNT odorant exposure only.

In the classical conditioning paradigm, the natural effects of EBS on the central nervous system acted as the unconditioned stimulus, with TNT odorant as the conditioned stimulus. Even in operant conditioning the rat was being classically conditioned. Because EBS makes EEG recording impossible, the EEG was recorded during extinction only. Recording sessions were interposed between EBS training trials to insure that extinction did not occur.

Results

Phase I

In the first phase twenty-three rats were subjected to the conditioning sessions. Of the 22 rats whose brain waves were analyzed, all showed conditioning of the brain wave activity to the TNT. In addition, one rat alone was trained, successbully, only to bar-press.

The criteria for success included comparisons of the EEG before and after conditioning, and, before and after the presentation of TNT and control odorants. The number of sessions required to produce the learning ranged from 3-16 sessions with a mean of 7.5 sessions. The sessions varied in length and number of trials, but an average session lasted approximately 30 minutes and included about 30 TNT and 30 non-TNT control trials.

Phase 2

The second phase concerned the intensive classical conditioning of five rats. In this study, cortical frequency spectra were recorded from four different brain sites: occipital, parietal, cingulate, and MFB. These EEG data were analyzed by means of the Fast Fourier Transformation (FFT) for seven bands: 1-3.5; 3.51-7.5; 7.51-12.5; 12.51-19; 5; 19.51-25.5; 25.51-35.5; and 35.51-45 Hz.

Three basic forms of analysis were employed: (1) comparison of the cortical frequency spectra, CFS, obtained during the base-line (prior to training) brain response versus TNT vapor brain response; (2) immediate pre-TNT brain response versus TNT vapor brain response, and (3) TNT vapor brain response versus control-odor brain response.

All five rats demonstrated some changes in their CFS to TNT vapor which differed from: baseline brain recordings, pre-TNT brain recording, and/or the control odor CFS. Some rats showed all changes.

Some rats showed pervasive changes in all four sites to TNT vapor stimulation, others showed more restrictive changes. Overall, the site in which the CFS changed most consistently and most often was the cingualte gyrus.

In general, there was a fairly pervasive change among the seven frequency bands studied. The most consistent and most frequent bands to show change were 0.5-7.5 Hz and 25.5-45.5 Hz.

During the first phase various parameters of testing were quickly investigated. The most important training parameter was found to be the "crispness" of the olfactory stimulus. That is, the stimulus onset and offset had to be made clearly evident to the rat before training was evident. Because olfactory stimuli tend to be vague, a special effort was made to eliminate stray TNT odors. (See Fig. 4) The olfactory stimuli were presented by a push-pull system which minimized the contamination of the environment with TNT vapors. Additionally, a vacuum was created inside the chamber near the testing cage to eliminate any unwanted vapors. Table 2 presents the results of this first phase of study, including rats which were not exposed to optimal parameters of conditioning. The probabilities in the table refer to the Chi Square Staaistic for the behavioral data. For the brain conditioning, the percentage time in spectral frequency was compared either immediately before or immediately after olfaction for control versus TNT odorants, or control and TNT odorants during trials were compared to pretraining norms. A rat was considered conditioned if the TNT comparisons were significantly different from the control odorant comparisons. The Chi Square for the behavioral data was based upon the four cell frequency of response table with headings "given TNT," "given nonTNT," "responded" and "failed to respond."

Five of the seven rats showed evidence of being behaviorally conditioned. No rat was run more than 53 sessions before conditioning was evident. Typically, it was the rats with high rates of self-stimulation which were conditioned most rapidly.

During the second phase of this study, parameters specifically dealing with classical conditioning were employed. New analyses were used, and various brain sites were explored. Phase one had investigated only transcortical electrode placements (anterior lateral to parietal contralateral); in phase two, occipital, parietal, cingulate, and medial forebrain bundle, (MFB) sites were investigated. Instead of analyses of spectral patterns, power in band was computed before training and pre- and post both for TNT and non-TNT odorant trials.

Method of Procedure

Surgical procedure. The operation employed was more complex than the previous one in that, in addition to a stimulating electrode in the medial forebrain bundle (MFB--the positive reward center), we implanted epidural electrodes in the parietal area, occipital area, and in the cingulate area. The electrodes in all four sites (i.e., the above three epidural sites and the subcortical site in the MFB) were referenced to an indifferent ground in the nasal region of the rat, providing four "monopolar" recordings. Five rats were so prepared.

The electrode implantation was accomplished by means of a Kopf Stereotaxic device which enabled us to locate the appropriate areas for the electrode tip. The coordinates are determined from atlases which were created from studies of hundreds of animals controlled for weight. The electrode used for MFB stimulation was bipolar, with the tips of the wires bared. The epidural electrodes were the ends of stainless steel screws which were attached to the skull. The top of the skull was covered with dental acrylic cement producing an "electrical mound," keeping the electrodes mechanically in place and insulating them.

The leads from the electrodes were connected to a rotary gold-ring commutator, allowing the animal to move within the Skinner Box without excessive encumbrance from the lead wires.

Conditioning procedures. Prior to classically conditioning the rat to the odor of TNT, they were all "shaped" to respond to a stimulus in the MFB by pressing a bar to deliver an electrical brain stimulus (EBS). By varying two factors of the EBS systematically (see Fig. 2) we determined the optimal characteristics of the EBS. This optimal state was defined as the maximum rate of bar-presses per minute.

The EBS consisted of a series of square waves of "y" voltage, "x" half-wave duration, and "Z" total train duration; x was kept constant at 0.2 ms; y was varied from 100 μV to 500 μV; by varying from 100 ms to 500 ms we essentially provided varying numbers of the "positive-zero-negative" complex of waves.

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Appendix 1 Summary of Phase One Results

KEY

S: subject number

Type: B = behavioral conditioning

C = classical conditioning

Status: B = Behaviorally Conditioned

C = Classically Conditioned

E = Electrical connection problems in rat kept rat from completing

D = Death due to toxic or other interference

NC = Not Conditioned

Level: Most significant probability that learning was not due to mere chance. If two values are presented, the first is for the behavioral data, the second for the classical conditioning.

NS = Not significantly different from chance. "_" not performed.

Test: Statistical Tests used to determine level (Behavioral, Classical)

C = Correlation

CS = Chi Square

FA = Friedman Analysis of Variance

Stimulation: Parameters of EBS used: rate per min.: μ V x msec.

Appendix 1 (continued)

S	TYPE	STATUS	LEVEL	TEST	STIMULATION
0	B	B	.001	CS	35;300x250
1	-	E	-	-	-
2	-	E	-	-	-
3	B,C	E	NS, none	CS	16;250x250
4	B,C	B,C	.001, .053	CS, FA	195; 175x200
5	B,C	C	NS, .001	CS, C	40; 475x250
6	B,C	B,C	.1, .005	CS, C	25; 600x500
7	B	D	.1	CS	115, 325x250
8	B	E	-	-	32; 600x250
9	-	E	-	-	18; 250x250
10	-	E	-	-	53; 225x250
11	-	-	-	-	Adversive to EBS
12	C	D	-	-	122; 225x250
13	-	E	-	-	43; 350x250
14	C	C	.005	C	45; 225x250
15	B	E	-	-	116; 350x250
16	C	C	.01	C	26; 400x250
17	C	C	.01	C	97; 200x250
18	-	-	-	-	154; 275x250
19	C	C	.005	C	108; 275x250
20	B,C	B,C	.001, .001	CS, C	130; 300x250
21	C	C	.005	C	30; 450x250
22	C	C	.025	C	131; 225x250

Appendix 1 (continued)

S	TYPE	STATUS	LEVEL	TEST	STIMULATION
23	C	C	.005	C	142; 275x250
24	B	D	-	-	47; 500x250
25	-	E	-	-	-
26	B	D	-	-	119; 225x250
27	-	D	-	-	-
28	-	D	-	-	-
29	C	C	.01	C	60; 225x250
30	C	C	.001	C	138; 250x250
31	C	E	-	-	57; 350x250
32	B	E	-	-	-
33	C	C	.001	C	112; 275x250
34	C	C	.05	C	38; 400x250
35	C	C	.05	C	90; 200x250
36	C	C	.05	C	67; 325x250
37	C	C	.05	C	31; 425x250
38	C	D	-	-	89; 175x250
39	C	C	.005	C	50; 200x250
40	C	C	.001	C	78; 175x250
41	C	C	.01	C	42; 400x250
42	C	NC	NS	-	70; 300x250

Appendix 2

The Faraday cage was constructed as follows:

A large (2'6" x 3'3" x 2'8") box was wrapped with copper foil except for a small rectangular opening on the top (10" x 9") which was covered with double copper screening thus allowing visual access to the closed box if a light were on in the box. All joints were soldered, and friction was used to hold the copper foil to the box frame. The top of the box was hinged, thus providing access. The box was also lined on the inside with copper foil, electrically insulated from the outside foil layer. Stainless steel-mesh (MGTSY) gaskets provided high-conductivity electrical contact from the top of the box to the rest of the box. The inner copper lining was electrically connected to the outer copper lining at one point by a soldered heavy gauge copper wire. Two identical steel alloy connector boxes were soldered to the box: one on the top and the outer copper wall, one on the top, inside the box, on the inner copper wall. Six BNC and six 1/4" plastic tubes connections could be made across the copper walls. On the copper screening an exhaust air hose was connected on either side providing negative air pressure at the bottom of the chamber. This negative pressure was useful both in eliminating stray odorants and in insuring proper ventilation.